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EXPERIMENTAL ARTICLES

Comparative Molecular Biological Analysis of the Microbial Community of the Holocene and Pleistocene Deposits of Posol'skaya Shoal, Lake Baikal

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Abstract—The bacterial diversity was studied in sediment layers of Posol'skaya Shoal station (Southern Baikal) belonging to different periods. A set of primers specific to individual bacterial groups was used to analyze the 16S rRNA gene fragments. The bacterial diversity in the Holocene deposits was found to be higher than in the Pleistocene ones. In the upper sediments, a positive PCR reaction with bacterial primers and with specific cyanobacterial and archaebacterial primers was detected. The following phylogenetic groups were revealed in the microbial community of the surface horizon: green nonsulfur bacteria, δ-proteobacteria, β-proteobacteria *(Nitrospirae*), α-proteobacteria, acidobacteria, crenarchaeota, euryarchaeota, and groups of uncultured bacteria. From the DNA of the Pleistocene deposits, the PCR product was obtained only with bacterial primers. The representatives of the genus *Pseudomonas* were most closely related to the sequences obtained (95–97% homology).

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Key words: Baikal, microorganisms, Pleistocene, Holocene, deposits, 16S rRNA.

The bottom sediments of Lake Baikal are a valuable source of data on the geological and climatic history of Siberia. Due to its ancient age and to the existence of regions of undisturbed sedimentation, this lake is especially attractive and useful for the reconstruction of the climates of the past [1]. The Posol'skaya Shoal station is one of the areas where the level relief indicates the presence of well-pronounced layers of Holocene deposits [2, 3]. Since the absence of disagreements in the seismic records on its northeastern slope indicates normal sedimentation over a long time interval, an uninterrupted sediment log can possibly be obtained there. This suggestion is supported by the data on the diatom content in Baikal bottom sediments; the diatoms are the main signal of the paleoclimatic chronicle [4]. Their distribution has been found to change synchronously with the global climate; their absence indicates flow termination during glaciations, which blocked the influx of silica required for the diatom development. The relations between microorganisms and the fluctuations of the paleoclimate is not yet clear, although the presence of prokaryotes in 16-Ma-old bottom sediments has been demonstrated by molecular techniques [5, 6]. Although the increase in prokaryote numbers with depth is less pronounced in deep-water sediments, their number and activity correlate with repeated layers of diatom sediments (ca. 9 Ma) [7].

The goal of the present work was to compare by molecular methods, the microbial diversity of Lake Baikal bottom sediments formed at different periods, Holocene and Pleistocene. The efficacy of this approach has been previously confirmed in studies of the presence and diversity of microorganisms in the sediments and water column of Lake Baikal [8, 9].

MATERIALS AND METHODS

Sampling. The samples were collected with a large gravity tube (5 m) in the course of the summer 2001 expedition of the *Vereschagin* research ship. The sampling was performed on the gentle northwestern slope of the Posol'skaya Shoal, at the depth of 200 m, at a significant distance from the top. The core was taken in such a way that the slope surface, where landslide bodies were revealed, was not sampled. The length of the core was 360 cm. Lithologic and diatom analyses were performed

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with a 10–20 mm step in order to control the integrity of the layers. After retrieval, the core was opened as quickly as possible. The internal part of one half of the core was placed in sterile bags and stored in liquid nitrogen prior to the molecular investigation under laboratory conditions. The DNA isolation, amplification, cloning, and sequencing conditions have been described previously [9]. The sequences were deposited in GenBank and are stored under the following accession numbers: AY839107–AY839135, AY833517–AY833524, and DQ178146–DQ178162. The second half of the core was used for diatom analysis and for the investigation of the physicochemical properties. The age of the core was determined according to the predominant algal complex.

Primers used in the work. The following primers complementary to bacterial 16S rRNA gene fragments: bacterial, 500L (CGTGCCAGCAGCCGCGGTAA) and 1350R (GACGGGCGGTGTGTACAAG) [10]; archaebacterial, Ar20F (TCCCGGTTGATCCYGCCRG) and Ar958R (YCCGGCGTTGAMTCCAATT) [11]; and cyanobacterial, CYA 106F (CGGACGGGTGAGTAACGCGTTA) and CYA 781Ra (GACTACTGGGGTATCTAATCCC(A/T)TT) [8].

RESULTS AND DISCUSSION

The bottom sediments collected at Posol'skaya Shoal station consisted of diatom-rich Holocene deposits and of Pleistocene clays with low diatom content (Fig. 1). The diatom shell composition of the Holocene deposits of the sampling region was mostly similar to that of the modern diatom flora. The distribution of the predominant species within the sediment layer was close to the typical distribution for undisturbed sedimentation. This is confirmed by the regularity in the ratio of predominant species; i.e., *Aulacoseira* and *Cyclotella* predominate in the upper sediment layers; *Cyclotella* lies below; and *Cyclotella*, *Aulacoseira, Stephanodiscus*, and *Synedra* prevail in still deeper layers. The Pleistocene sediments contained a small amount of diatom shells (Fig. 1), with a noticeable peak of diatom development at a depth of 120 cm, coinciding in time with the short-term Belling warming.

The bacterial cells, as revealed by DAPI staining, were found in all the layers of the core. Their numbers in the surface layer and in the 357–359 layer were $42.5 \pm 2.1 \times 10^6$ and $13.8 \pm 0.7 \times 10^6$ cells/g wet silt, respectively.

Total bacterial DNA was isolated from these sediment layers and amplified with various primers. The results of the PCR analysis are presented in Table 1.

PCR products were obtained for only some of the primers. A positive result with bacterial, archaeal, and cyanobacterial primers was obtained with the DNA from the surface layer of the bottom sediments. For the DNA from the deep horizon, products were obtained only with bacterial primers. In all other cases, the result was negative, although various reaction conditions were tested.

Table 1. Results of the PCR analysis of the total DNA from different layers of the bottom sediments of Posol'skaya Shoal station and the number of clones obtained

Depth, cm	Bacterial primers	Cyanobacterial primers	Archaebacterial primer
1–3	+ 99 clones	+ 42 clones	+ 54 clones
357–359	+ 56 clones	–	–

The phylogenetic analysis of the sequences obtained from different horizons revealed significant differences in the composition of the microbial community of the surface and deep-water sediments (Fig. 2–6). The similarity ratio varied from 87 to 99%.

The sequences obtained from the surface sediment layer with bacterial primers were assigned to the following taxonomic groups: green nonsulfur bacteria, δ-proteobacteria, β-proteobacteria (*Nitrospirae*), and α-proteobacteria (Table 2). The taxonomic placement of some sequences (AY839122, AY839108, and AY839115–AY839119) was not defined, since the closest homologues were uncultured bacteria with unclear phylogenetic position (Fig. 2). The closest homologues were found in Indian soils, in sediments of a number of lakes and oceans, and in hydrothermal springs.

The species diversity of the Pleistocene sediments was lower. The sequences obtained from the DNA of deep-water sediments were closest to the sequences of the genus *Pseudomonas* (95–97% similarity). They formed an isolated cluster on the phylogenetic tree (Fig. 3). We have already reported sequences close to *Pseudomonas* in the deep-water sediments containing gas hydrates [9]; these sequences, however, were not identical. The closest homologues of the Baikal sequences were detected in Arctic ice, in an ancient ice core from China, and in various water samples. As has already been mentioned, this horizon was formed during a cold period and contains practically no diatoms (Fig. 1).

Analysis of the clones from Holocene deposits with archaeal primers using BLASTN search engine revealed low similarity with the GenBank database sequences (Table 2). The sequences were most closely related to the ones obtained from deep-water sediments and hot springs. Seven sequences belonged to the cluster of uncultured *Crenarchaeota.* Six sequences

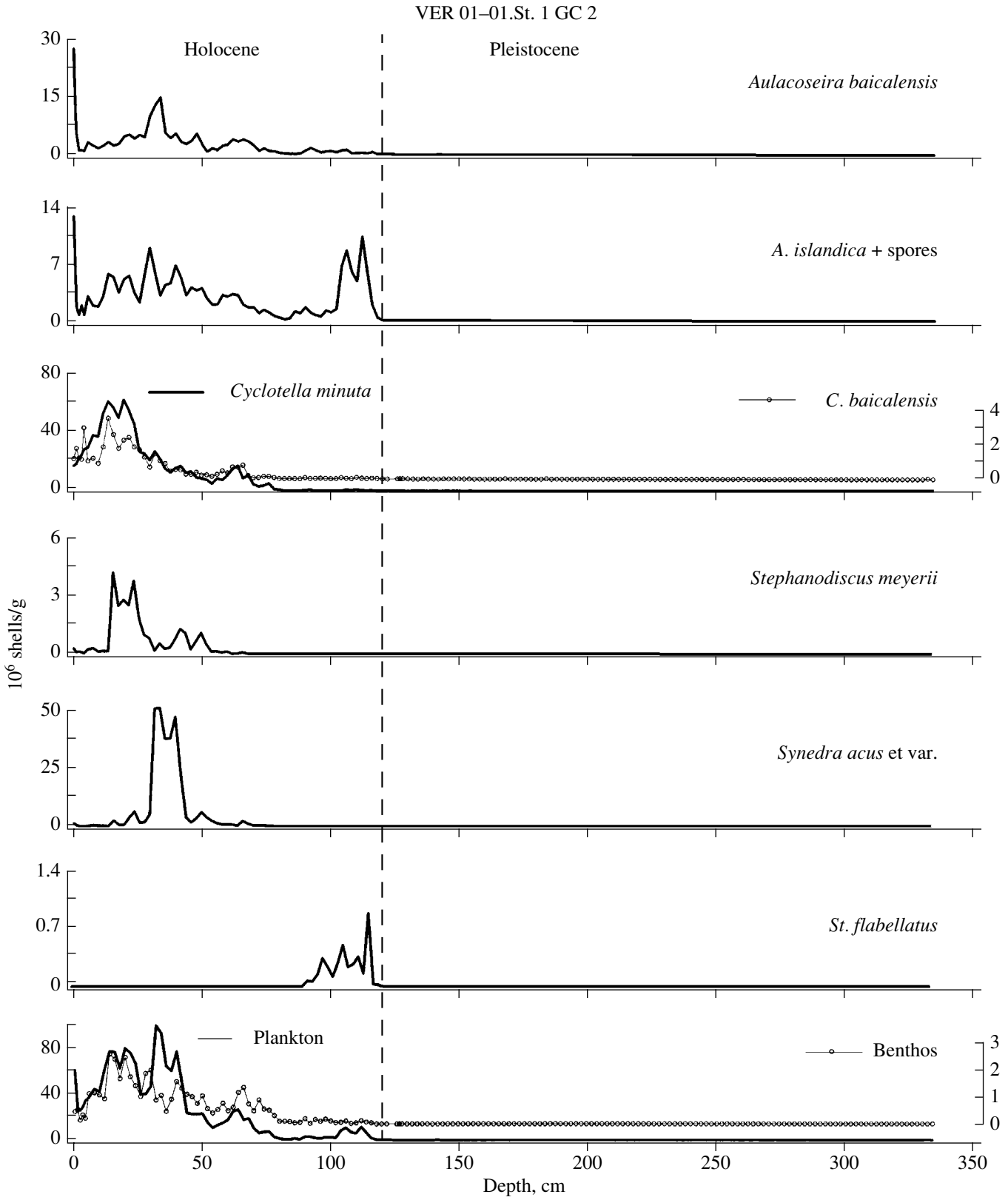

Fig. 1. Distribution of diatom shells (10^6 shells per 1 g dry sediment) in Lake Baikal sediments (VER 01-01, St. 1 GC-2).

Table 2. Closest homologues of the Baikal sequences

	Phylogenetic group	Clone	Closest homologue	%
Universal bacterial primers	δ-proteobacteria	16 1-3	uncultured bacterium AY427825	96
		3 1-3	uncultured bacterium AY427825	95
		25 1-3	uncultured bacterium AF424254	91
	β-proteobacteria	8 1-3	clone from saline soil LCP-6 AF286037	94
	α-proteobacteria	5 1-3	uncultured α-proteobacterium AF509580	98
		2 1-3	uncultured bacterium AY186071	98
	Green nonsulfur bacteria	22 1-3	uncultured bacterium AY093480	96
	Bacteria	C 1-3	uncultured soil bacterium AY289463	97
		23 1-3	uncultured soil bacterium PRR-7 AJ390478	93
		20 1-3	uncultured bacterium AB116391	91
		24 1-3	uncultured bacterium AF407700	99
		19 1-3	uncultured δ-proteobacterium AY083025	94
		17 1-3	uncultured bacterium AF429021	94
		21 1-3	uncultured bacterium AF443574	95
		13 1-3	uncultured bacterium AJ536836	99
		11 357-359	*Pseudomonas* sp. U85870	92
		24 357-359	uncultured bacterium AY212724	97
		7 357-359	uncultured bacterium AY212623	96
		18 357-359	uncultured bacterium AY212623	95
		13 357-359	proteobacterium BHI80-88 AJ431228	98
		19 357-359	*Pseudomonas* sp. AY214345	95
		17 357-359	bacterium from Arctic ice AF468404	98
		14 357-359	*Pseudomonas* flavescens AJ308320	96
		22 357-359	uncultured α-proteobacterium AF509580	98
		21 357-359	uncultured bacterium AY321382	95
		15 357-359	uncultured bacterium AF328183	90
		25 357-359	*Pseudomonas* sp. PH10B AY649403	97
Archaebacterial primers	Euryarchaeota	54 1-3	uncultured archaeon AY555830	97
		25 1-3	uncultured bacterium AF540863	91
		56 1-3	uncultured crenarchaeon AJ567628	96
		43 1-3	uncultured *Methanosaeta* sp. AY177808	89
	Crenarchaeota	40 1-3	uncultured crenarchaeon AJ567628	95
		19 1-3	uncultured crenarchaeon AJ567640	95
		27 1-3	uncultured crenarchaeon AJ567640	95
		48 1-3	uncultured archaeon AY354118	92
		17 1-3	uncultured archaeon AY354118	92
		21 1-3	uncultured archaeon AY555819	97
		39 1-3	uncultured archaeon AB161341	97
Cyanobacterial primers	*Synechococcus*	24 1-3	*Synechococcus* sp. AY151248	99
	Acidobacteria	2 1-3	uncultured acidobacterium AY177760	92
		26 1-3	uncultured acidobacterium AY177760	96
		20 1-3	uncultured bacterium AF523991	96
	Bacteria	15 1-3	uncultured bacterium AY768982	85
		22 1-3	uncultured bacterium AY781375	98
		23 1-3	uncultured δ-proteobacterium AF424217	96
		28 1-3	uncultured bacterium AY453255	98
		27 1-3	uncultured acidobacterium AY689603	97
		11 1-3	uncultured bacterium AJ607258	93
		32 1-3	uncultured bacterium AY160876	96

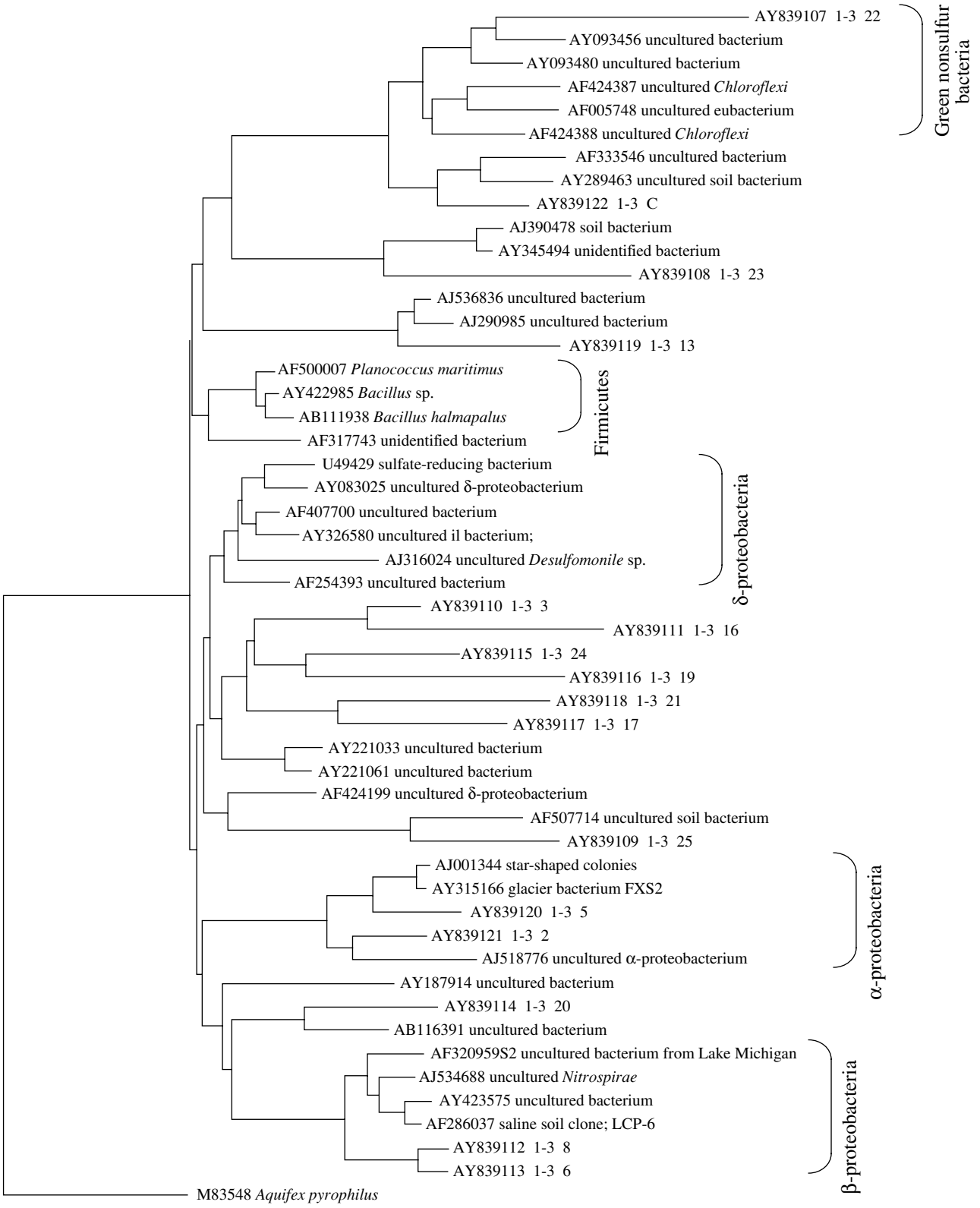

Fig. 2. Phylogenetic position of the bacteria recovered from Lake Baikal sediments, Posol'skaya Shoal station, 1–3 cm.

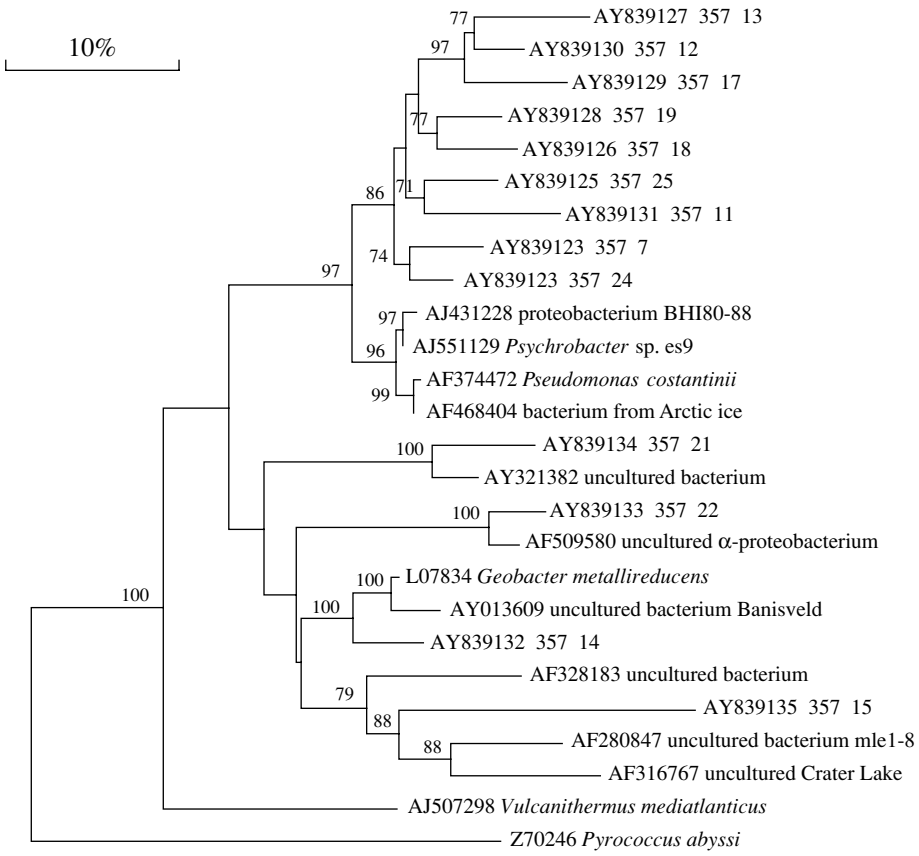

Fig. 3. Phylogenetic position of the bacteria recovered from Lake Baikal sediments, Posol'skaya Shoal station, 357–359 cm.

belonged to *Euryarchaeota.* Four of them formed an isolated cluster on the phylogenetic tree (Fig. 4). This cluster occupies the position between the clusters ANME-1 and ANME-2, which include archaea participating in anaerobic methane oxidation [12]. More detailed analysis revealed that clone 54 belonged to the cluster of uncultured *Methanosaeta* (Fig. 5) which participate in methane synthesis [13]. The homology was 98%.

A library of 42 clones was obtained with specific cyanobacterial primers; 14 of them have been analyzed. Two sequences were represented by two and three clones. One sequence (24) showed 99% similarity with *Synechococcus* sp. (*Cyanobacteria*). Representatives of this genus are widespread in Lake Baikal [14]. They were previously revealed in Lake Baikal water column on the basis of their morphological features and 16S rRNA gene analysis. The emergence of planktonic species, including cyanobacteria, in the DNA extracted from the upper bottom sediments is not surprising, considering that depths in the vicinity of the Posol'skaya Shoal do not exceed 200 m. The cyanobacterial sequence obtained from the sediments is close to the cyanobacterial sequences obtained from the water column of Lakes Biva and Nagasaki (Japan); Lake Constanz (Central Europe); and Lakes Maggiore (Italy), Mondsee, and Hallstatersee (Austria) [15].

The primers used for the analysis of cyanobacteria are not strictly specific and also reveal bacteria from other phylogenetic groups. One sequence belonged to δ-proteobacteria and three to *Acidobacteria.* All the sequences are most closely related to the sequences obtained from soils and marine sediments. Two sequences belong to the two cluster groups OP10 and JS1 (Fig. 6); these groups are revealed in deep-water marine sediments [16].

The results of biodiversity studies of environmental microbial communities by molecular techniques can be affected by several factors. For instance, DNA extraction from bottom sediments can occur concomitantly with the coextraction of PCR inhibitors [17]. Therefore the reagents and procedure described by Rochelle et al. [17] was used for DNA extraction from Baikal sediments in order to avoid the interference by humic acids and to obtain purer DNA sam-

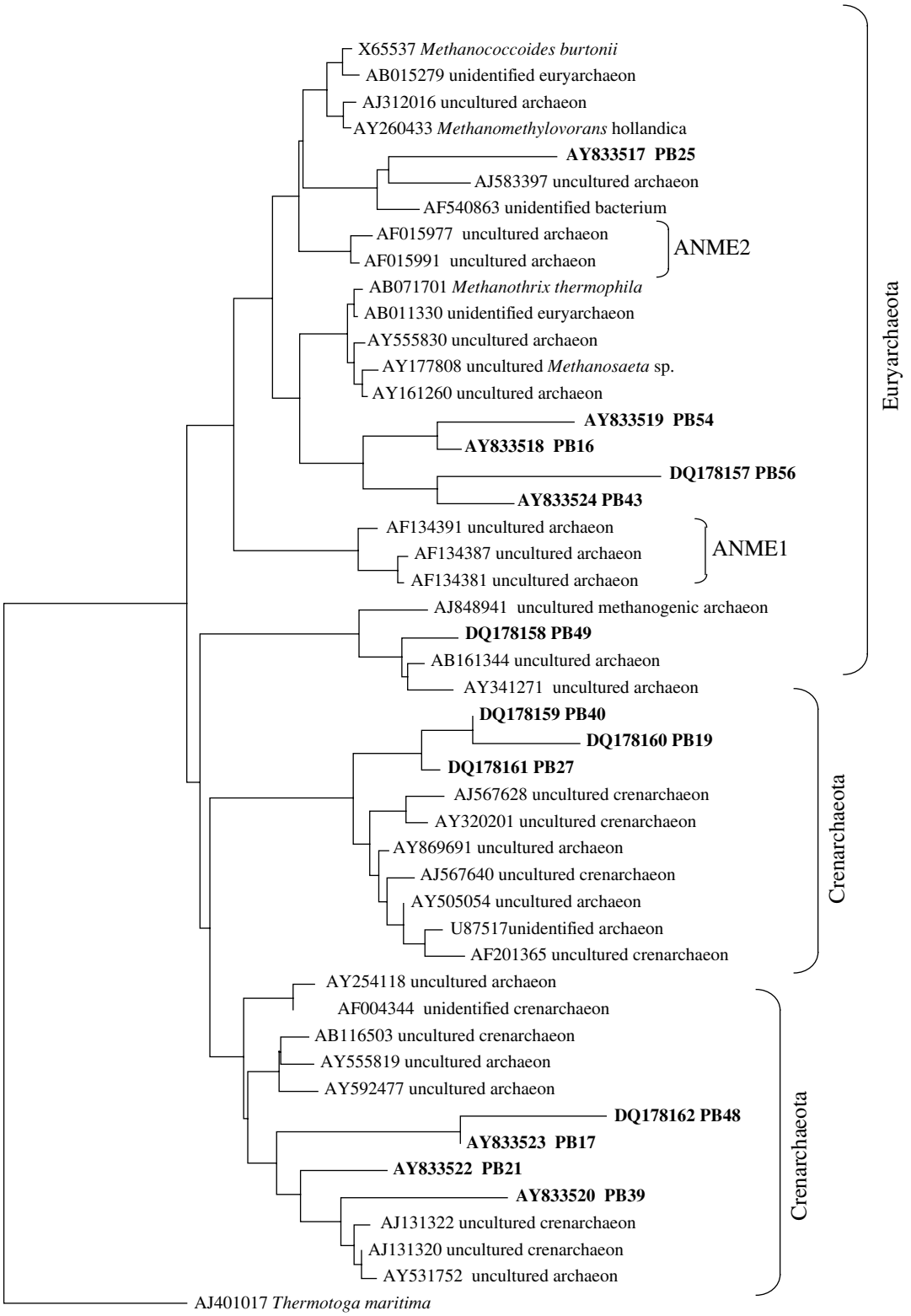

Fig. 4. Phylogenetic position of the archaea recovered from Lake Baikal sediments, Posol'skaya Shoal station, surface horizon.

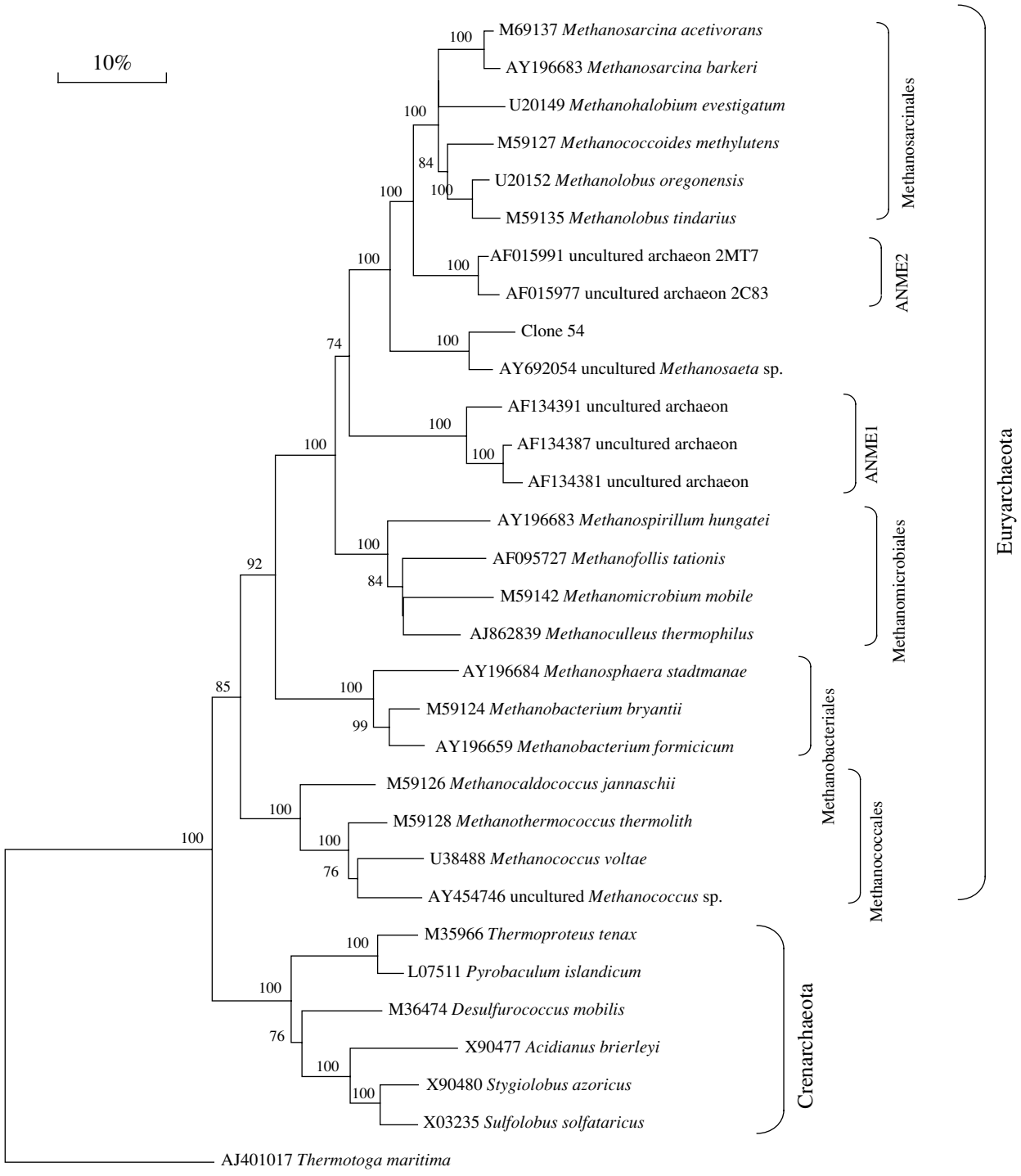

Fig. 5. Phylogenetic position of the archaeal clone 54.

ples. The presence of *E. coli* sequences and other contaminants in *Taq* polymerase can result in a biased picture of microbial diversity [18, 19]. In order to broaden the spectrum of the sequences revealed in the Holocene sediment, other primers, aside from bacterial primers, were used in this work. The absence of positive PCR with group-specific primers for the DNA from ancient clayey sediments

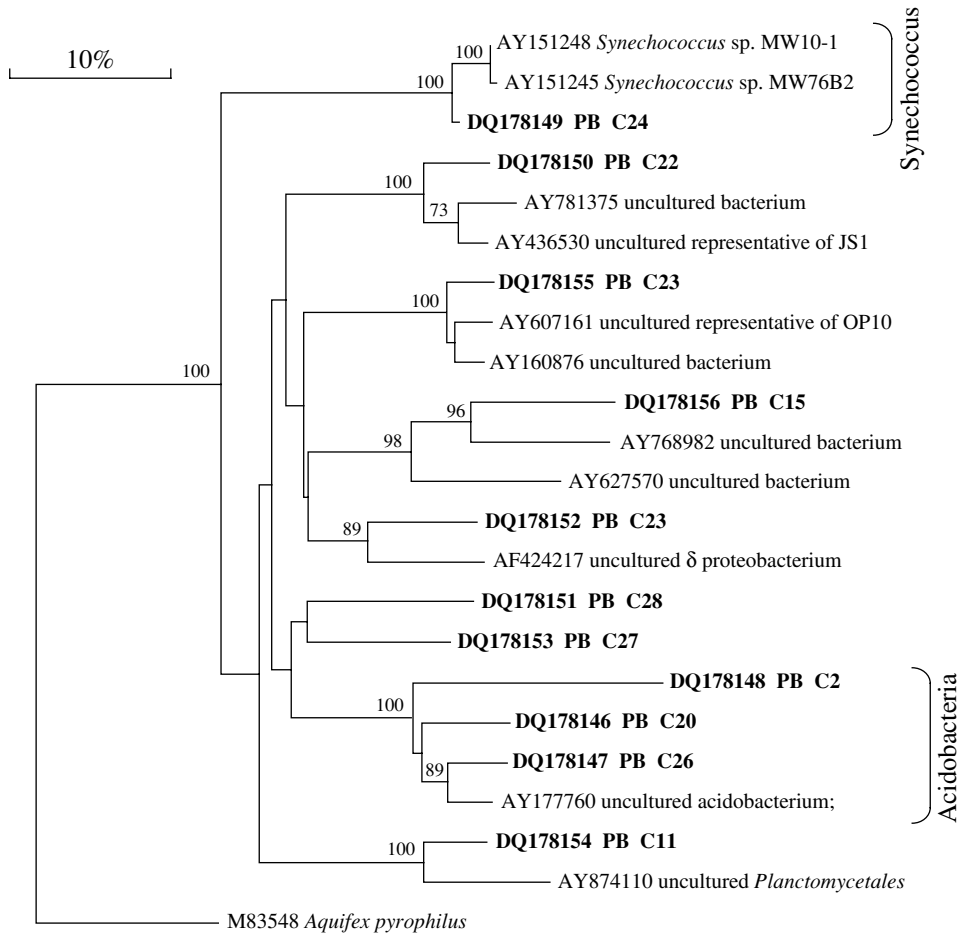

Fig. 6. Phylogenetic position of the bacteria recovered from Lake Baikal sediments with cyanobacterial primers, Posol'skaya Shoal station, surface horizon.

indicates the less diverse composition of the microbial community. The surface sediments contained a broad spectrum of species belonging to different phylogenetic groups.

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